

I, PAUL ANDREW BENJES, hereby declare the following:

1. I am currently the Process Development Manager for GlycoSyn^{IRL}, a business unit of Industrial Research Limited, specializing in the process development and scale-up manufacture of small molecule organic APIs for human clinical trials. I hold a PhD in Chemistry from University of Otago, New Zealand, and have been an active synthetic organic chemist for the past 17 years. My Curriculum Vitae sets forth further details of my research and educational background (Exhibit A).
2. I received a BSc(Hons) in Chemistry from University of Otago in 1987, and PhD in Organic Chemistry from University of Otago in 1994.
3. I am one of the named inventors in the present application. The invention described in the subject application pertains to a process for the preparation of an intermediate useful for preparing kifunensine. The invention also relates to a process for preparing kifunensine.
4. I understand that claims 1, 8-9, 11-17, and 19-23 of the application have been rejected as being unpatentable over Kayakiri *et al.* *Chem. Pharm. Bull.* **1991**, *39*, 1392-1396 ("Kayakiri I reference"), and Kayakiri *et al.* *Tetrahedron Lett.* **1990**, *31*, 225-226 ("Kayakiri II reference").
5. Under my direction, attempts to prepare kifunensine on a large scale using the methods of Kayakiri were made. The specific procedures followed, as based on the Kayakiri references, are described in Exhibit B, attached hereto, entitled "REPORT ON THE ATTEMPTED REPLICATION OF KIFUNENSINE SYNTHESIS USING THE METHODS OF KAYAKIRI, AND SUBSEQUENT INVESTIGATIONS USING N-ACETYL-D-MANNOSAMINE".
6. As described in the attachment, the method of Kayakiri *et al.*, when used to prepare kifunensine on a large scale, suffered from irreproducibility in the silylation step, which resulted in a lower overall yield than that reported by Kayakiri *et al.* Kayakiri *et al.* initially introduce an N-oxamoyl group that forms part of the final product, kifunensine. In our view, the problems of irreproducibility in the silylation step arise from the carry-over of reagents from the initial step that introduces the N-oxamoyl group. According to the Kayakiri *et al.* procedure, the N-oxamoyl group is never removed. In contrast, the present invention focuses on the use of an N-acetyl protecting group, which is removed after silylation. The removal of the N-acetyl group requires the cleavage of an amide group under quite harsh reaction conditions, namely the use of a strong base at elevated temperature. It is only after removal of the N-acetyl group that the N-oxamoyl group is introduced.
7. As noted above, the difference between the present invention and the Kayakiri *et al.* procedure lies in the use of the N-acetyl protecting group. Indeed, we spent more than two years endeavoring to reproduce the Kayakiri *et al.* method on a large scale, before the inventive idea occurred to us. Even then, it was surprising to us that it was so successful. It could not be predicted that switching from the N-oxamoyl protecting group to an N-acetyl group would improve the selectivity and reproducibility of the 6-O-silylation step. Also, it could not be predicted that the conditions used to cleave the N-acetyl amide bond (strong base/elevated temperature) would leave the acetal protecting groups intact.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

12 December 2005
Date

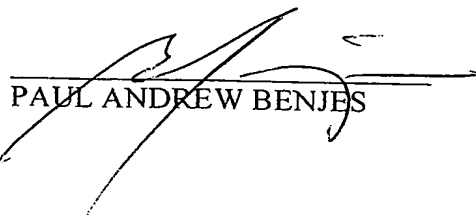

PAUL ANDREW BENJES

EXHIBIT A

CURRICULUM VITAE

Paul Andrew Benjes

Nationality : New Zealander
Place of Birth : Hong Kong
Date of Birth : 12 January 1967
Marital Status : Married

Address : 15 Fantail Grove
Belmont
Lower Hutt
New Zealand

Telephone : +64 4 9313224
Fax : +64 4 9313497
E-mail : p.benjes@glycosyn.com

Education :

1979 - 1983	<u>Hutt Valley High School, Lower Hutt</u> A Bursary
1984 - 1987	<u>University of Otago, Dunedin</u> BSc (Honours), Second Class * (Double major in Biochemistry). Honours papers in Organic, Physical and Inorganic Chemistry. Honours project on substitution reactions of heteroaromatics.
	*Medical certificates submitted for impairment.
1988 - 1994	<u>University of Otago, Dunedin</u> PhD. Under the supervision of Assoc. Prof. M.R. Grimmett.
	Research topic : N-Alkylation of Imidazoles Structurally diverse unsymmetrical imidazoles were synthesised for the purpose of examining the

regioselectivity of N-alkylation; substituent, reagent and medium effects were investigated.

Publications :

N-Alkylation of Nitrogen Azoles

Benjes, P.A. and Grimmett, M.R. in *Advances in Detailed Reaction Mechanisms*, J.M. Coxon (ed.), JAI Press, USA, Vol. 3, 1994, pp. 199-250.

Alkylation of 4(5)-Substituted Imidazoles

Paul A. Benjes and M. Ross Grimmett, *Heterocycles*, **32** (2), 735 (1994).

Polymers and Oligomers with Transverse Aromatic Groups and Strongly Constrained Chain Conformations

Roger W. Alder, Kevin R. Anderson, Paul A. Benjes, Craig P. Butts, Panayiotis Koutentis and Guy A. Orpen, *Chem. Commun.*, 1998, 309.

Royal Society of Chemistry, Specialist Periodical Reports, Carbohydrate Chemistry, Volume 34, Cambridge, 2003.

P. A. Benjes, R. Blattner, R. J. Ferrier, R.A. Field, R.H. Furneaux, C. Hamilton, J.O. Hoberg, K.P.R. Kartha, P.C. Tyler, and R.H. Wightman.

Inhibitors of ADP-Ribosylating Bacterial Toxins Based on Oxacarbenium Ion Character at their Transition States

Guo-Chun Zhou, Sapan L. Parikh, Peter C. Tyler, Gary B. Evans, Richard H. Furneaux, Olga V. Zubkova, Paul A. Benjes and Vern L. Schramm, *J. Am. Chem. Soc.*, **126**, 5690-5698 (2004).

Teaching Experience :

1988 - 1994

University of Otago

Laboratory Demonstrator for 1st, 2nd and 3rd year Chemistry.
Laboratory Supervisor for 1st year Chemistry (1990 - 1993).
Lecturer / Lab.Supervisor / Examiner for the Organic Chemistry component of the 1st year University course "Biochemistry for Physiotherapy" (11 lectures), 1991.

Private tutor in Chemistry and Biochemistry at the 1st and 2nd year levels.
Tutor to overseas students under the auspices of the Ministry of External Relations and Trade.

1995 - 1998

University of Bristol, England

Organic Chemistry workshops for 2nd and final year undergraduate students.

Employment :

1989 - 1993

House Tutor

University College, Dunedin.

Part-time employment as resident House Tutor at University College, a hall of residence housing 330 students. Duties included, *inter alia*, the implementation and maintenance of a comprehensive tutorial system, utilising both resident and external tutors. Extensive chemistry tuition was provided on my part, often to groups in excess of 60 students.

1988 - 1989

Undergraduate Laboratory Demonstrator

University of Otago

Laboratory demonstrator for 1st year general Chemistry; 2nd and 3rd year Organic Chemistry.

1990 - 1993

Laboratory Supervisor

University of Otago

Laboratory supervisor for 1st year general Chemistry.

1991

Lecturer in Organic Chemistry

University of Otago

Inaugural lecturer for the Organic Chemistry component of the 1st year University course "Biochemistry for Physiotherapy" (11 lectures). This course necessitated the preparation of new lecture and examination materials.

1995 – 1998

Post-doctoral Research Assistant (to Prof. Roger W. Alder)

University of Bristol, England

The principle areas of research include (i) the synthesis of a number of novel medium-ring carbobicycles with the intent of investigating intra-bridgehead interactions, (ii) the development of an anionic ring-opening polymerisation process for disubstituted cyclopropanes with the aim of generating novel polymers bearing considerable conformational control, and (iii) the synthesis of medium-ring bicyclic diphosphines and diamines as potential chelators in transition metal catalysis.

1998 – 2005

I. Research Scientist, Carbohydrate Chemistry Team

Industrial Research Limited, Wellington

Involved in the commercial contract synthesis and process development to multi-kilo scale of a wide variety of (potential) drug substances

including for example thioglycosides, lysine-based dendrimers, aza-sugars, and phosphorylated inositols. Developed and lead a Process Development Team.

2005 – present

II. Process Development Manager

GlycoSyn^{IRL}

Industrial Research Limited, Wellington

GlycoSyn^{IRL}, a Business unit of IRL, is a cGMP chemical manufacturer, specialising in the synthesis of small molecule API's for use in early phase human clinical trials. As PD Manager I am responsible for a Team of seven process development chemists and for the overall project management of various commercial cGMP API synthesis contracts.

Referees :

Dr. Richard Furneaux (Carbohydrate Team Manager)

Industrial Research Limited

PO Box 31-310

Gracefield

Lower Hutt, New Zealand

ph. : +64 4 569 0000

fax : +64 4 569 0055

e-mail : r.furneaux@irl.cri.nz

Prof. Roger W. Alder (Post-doctoral supervisor)

School of Chemistry

University of Bristol

Cantock's Close

Bristol BS8 1TS, England

ph. : + 44 117 928 7657

fax : + 44 117 929 8611

e-mail : rog.alder@bristol.ac.uk

Assoc. Prof. M. Ross Grimmett (PhD supervisor)

C/- Department of Chemistry

University of Otago

PO Box 56

Dunedin, New Zealand

ph. : (03) 479 7908

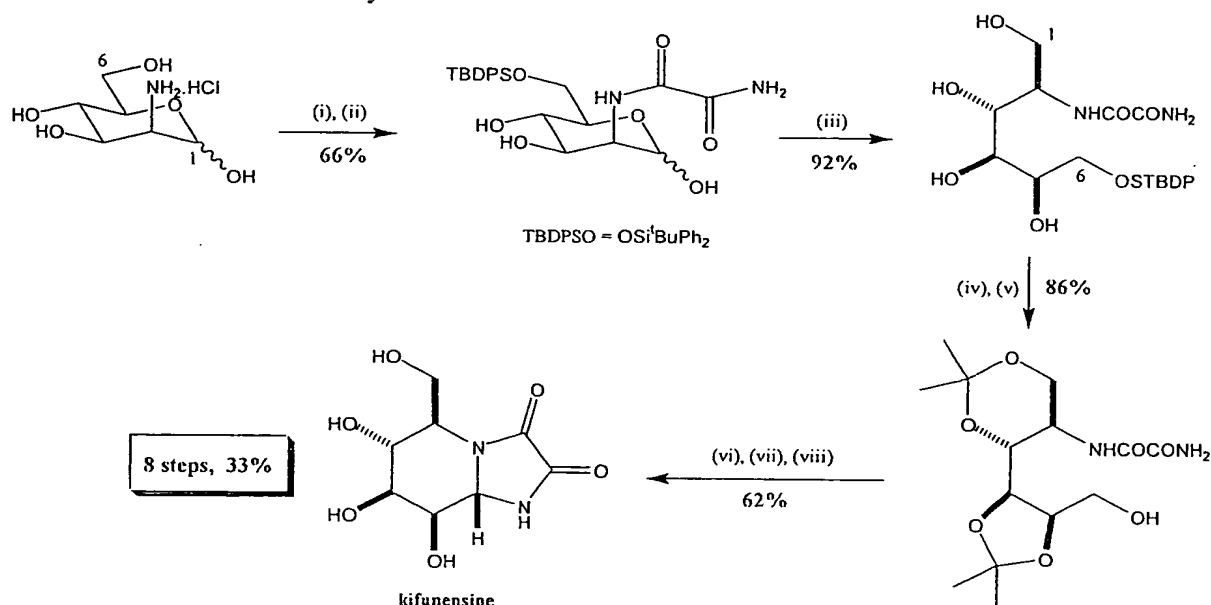
fax : (03) 479 7906

email : grimmett@alkali.otago.ac.nz

EXHIBIT B

REPORT ON THE ATTEMPTED REPLICATION OF KIFUNENSINE SYNTHESIS USING THE METHODS OF KAYAKIRI AND SUBSEQUENT INVESTIGATIONS USING *N*-ACETYL-D-MANNOSAMINE

Our initial efforts in the large scale synthesis of kifunensine were focused on the published route of Kayakiri *et al.* shown in **Scheme 1** (H. Kayakiri; C. Kasahara; K. Nakamura; T. Oku and M. Hashimoto, *Chem. Pharm. Bull.*, 1991, 39, 1392-1396). This route starts with commercially available D-mannosamine hydrochloride.



Scheme 1 - Reagents and conditions: (i), H₂NCOCOOH/DCC/HOBT/Et₃N/DMF; (ii), ^tBuPh₂SiCl/Im/DMF/0 °C; (iii), NaBH₄/MeOH/0 °C; (iv), Me₂CO/BF₃OEt₂/-20 °C; (v) ^tBu₄NF/THF/-20 °C; (vi), CrO₃·2Py/DCM; (vii), 7*N* NH₃-MeOH; (viii), TFA/H₂O.

The published yield for this route is 33%. However, our initial applications of the published route on a 100 g scale afforded only a 4% overall yield of kifunensine. We subsequently investigated modifications, generally involving minor alterations to reaction work-up and product isolation, but were only able to improve this overall yield to approximately 7-16% - less than half the percentage yield reported by Kayakiri *et al.* While endeavoring to optimize the process, we noted marked irreproducibility in the silylation step (Step (ii)), which means that the Kayakiri method does not respond well to scale-up. In particular, the ratio of mono-6-*O*-silylated- to 1,6-di-*O*-silylated products is highly variable and in our hands ratios ranging from 5:1 (in favor of the desired mono-silylated species) to as low as 2:1 are obtained. This variability is thought to be due to the unavoidable carry-over of reagents from the oxamic acid coupling reaction (Step 1).

A great deal of process development was carried out on this silylation step, aimed at reducing ratio and yield variability. This met with very little success. A number of alternative silylating agents such as TBDMSCl and TIPSCl, and reaction conditions exploring such factors as mode of

addition, temperature, solvent and the amount of silylating agent were trialed. The reproducibility of this step, however, was not markedly improved as a result of this work.

Purification of the precursor oxamide is problematic. The high aqueous solubility of the oxamide precursor precludes purification by selective solubilization and the material fails to crystallize/precipitate from the reaction or workup mixtures. Alternatives to the DCC/HOBt coupling methodology were also trialed, for example the use of CDI (carbonydiimidazole), but the purification problems remained and the subsequent silylation reactions were still highly variable in our hands.

During our investigation of the silylation step, we carried out trial silylations of *N*-acetyl-D-mannosamine (which is obtained as a monohydrate in pure crystalline form from the base-catalyzed epimerisation of *N*-acetyl-D-glucosamine). In contrast to the Kayakiri method, the procedure using *N*-acetyl-D-mannosamine allowed us to obtain very high yields of the mono-6-*O*-silylated derivative (*ca.* 9:1 mono- : di-*O*-silylated product) *reproducibly* irrespective of scale. At this point work changed focus from the Kayakiri *et al.* method to a route to kifunensine based on *N*-acetyl-D-mannosamine.